II. MATERIAL AND METHODS

MATERIAL AND METHODS

The state of Kerala, 'God's own land' lies in the south-west corner of the Indian subcontinent between north latitudes 8° 18′ and 12° 48′ and east longitudes 74° 52′ and 77° 22′ and covers 38,864 km^2 which is 1.2% of the geographical area of India and its population as per 1991 census is over 29 millions, making it one of the most densely populated regions of the world. It is among the wettest regions of the world, with an average rainfall of 2615mm. (Abbasi *et al.*, 1997).

The coastal plains of Kerala have about 34 backwater systems, of which the Vembanad Lake, south of Kochi is the largest one followed by Ashtamudi Lake further south. It is bounded in the east by Western Ghats and in the west by Arabian Sea (Sasidharan and Nagesh, 1998).

Geomorphologically, the central part of Kerala coast is mainly sandy. The coastal plain from Alapuzha to Kochi has a series of parallel to sub parallel sand dune ridges. It is the largest estuarine system on the west coast of India, having permanent sea connection at Kochi and Azhicode. All the rivers of Kerala are prone to salinity intrusion. During monsoon, there is inflow of large volumes of fresh water, pushing the salinity of the seawater westwards. Sea erosion on the coastal tract is a frequent feature of Kerala. In the later half of 19th century, vast stretch of forest land was leased out for commercial crops like tea, coffee, cardamom and rubber

cultivation. The real depletion commenced from here and continued unabated till 1980 (Sasidharan and Nagesh, 1998). Kerala is accessible to maritime influence from the west and has been important in history for mainly 2000 years.

Different parameters associated with the backwaters and the estuaries of Kerala have been reported by Qasim, 1973. During the southwest monsoon, almost fresh water condition persists throughout the estuary.

Though mangrove ecosystems occur in tropical and subtropical regions, the floristically richest mangroves are found in tropical southeast Asia (Linden and Jernlov, 1980; Dodd *et al.*, 1995). Mangroves are taxonomically diverse assemblage of woody plant communities belonging to several unrelated angiosperm families with special adaptations to saline condition (De Silva and Balasubramanian, 1985; Tomlinson, 1986). Although they are found in tropical areas world-wide, mangroves become more limited in their distribution in subtropical areas due to lack of low temperature resistance (Linden and Jernelov, 1980; Dodd *et al.*, 1995). The structure and species composition of the mangrove forest varies as a function of geographical, geophysical, geological, hydrographic, climatic and edaphic factors and man's activities (Yeragi, 2000). The identification of taxa, information on the geographic structure and the understanding of the organisation of the existing genetic diversity within and among populations are very important for the conservation of genetic resources

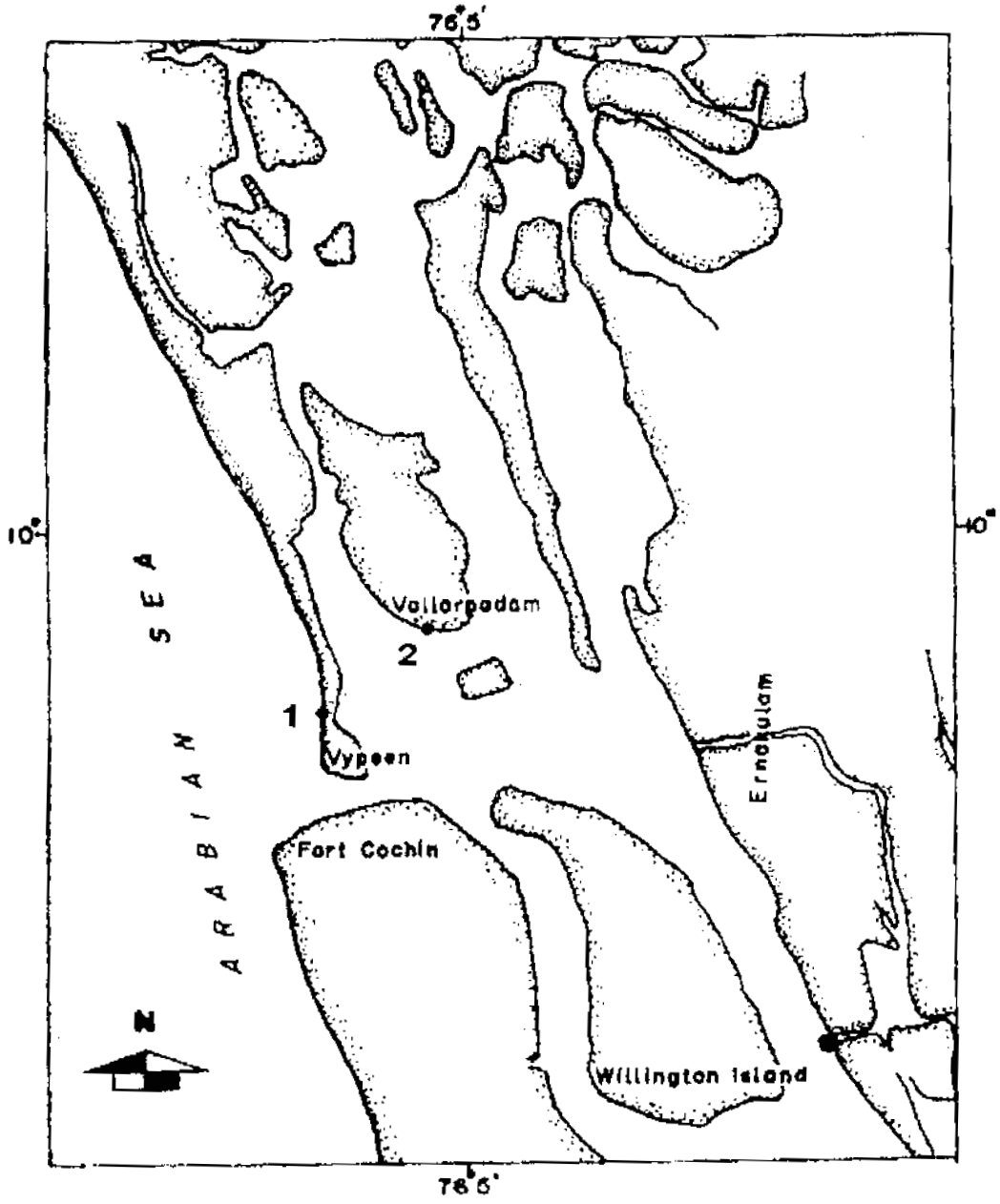

Fig. I. Map showing the sampling stations in the Cochin Backwaters.

1. Puduvyppu 2. Vallarpadam

(Triest, 1991; Aldrich *et al.*, 1992; Russel *et al.*, 1993; Dawson *et al.*, 1995; Dawson, 1996). Genetic and geographic variation of the mangrove tree *Bruguiera*, Lam. in Sri Lanka was studied by Pushpa Damayanthi *et al.*, (2000).

Prior to the commencement of the work, a preliminary survey was conducted in the backwater at Puduvyppu and Vallarpadam in the first fortnight of March 2000 to fix up the sampling stations and the time of collection for regular monthly sampling (Fig. 1). The first station is a typical mangrove ecosystem with a network of channels at Puduvyppu in Vypeen island and the second station is located near to the first is Vallarpadam, 3.5 km. away. A control station, which is the lower water of the rainbow bridge in the marine drive *ie.* at the mouth of the market canal was taken for the comparison of the hydrological parameters. The main species of mangrove plants in both these stations are *Acanthus ilicifolius* L., *Acrostichum aureum* L., *Avicennia officinalis* L., *Bruguiera cylindrica*, W.&A., *B.gymnorrhiza*, Lam., *Excoecaria agallocha* L., and *Rhizophora mucronata*, Lam. Floristic study of the mangrove ecosystem of the Mangalavanam, Kochi was studied by Sivadasan (1994).

The present study considered the following parameters:

A. VEGETATION STUDY

Regular field trips were made to different mangrove areas in different seasons in order to study the floristic composition and all the

available species of mangroves and their associates were collected from Puduvyppu and Vallarpadam for their systematic investigation.

B. FOLIAR EPIDERMAL STUDIES

a. Stomatal ontogeny

b. Stomatal index

c. Stomatal frequency

d. Palisade ratio

C. HISTOCHEMICAL ANALYSIS - QUALITATIVE AND QUANTIATIVE

a. Qualitative - Histochemical localisation of metabolites in foliar epidermis.

1. Starch
2. Insoluble polysaccharides
3. Sulphated and carboxylated polysaccharides
4. Polyphenols
5. Total proteins
6. Total lipids

b. Quantitative

1. Chlorophyll and carotenoids
2. Total carbohydrate
3. Total protein
4. Tannin
5. Moisture

6. Ash

7. Nitrogen

8. Sodium

9. Potassium

D. POLLEN MORPHOLOGY

a. Pollen size

b. Pollen shape

c. Exine thickness of pollen grain

d. Surface ornamentation of the pollen grain

E. WATER QUALITY PARAMETERS

The study was conducted in water-logged areas at Puduvyppu and Vallarpadam and the control station. In order to understand the seasonal and spatial variations of hydrological and biochemical parameters, the whole year was divided into three distinct periods namely-monsoon (June-September), post monsoon (October-January) and pre-monsoon (February-May). Five sites were selected in each station and the studies in the 3 stations were conducted every month for a year (April 2000 to March 2001). Monthly water samples were collected from each station and analysed for temperature, pH (Hydrogen ion concentration), salinity, dissolved oxygen, gross primary productivity and net primary productivity. The primary productivity varies considerably in the estuarine ecosystem in space and time.

F. ECONOMIC IMPORTANCE

The dense root-mat (mainly pneumatophores and stilt roots) characteristic of mangroves reduces the wave energy of the silt-laden waters and the fine particles of sediments get deposited and by virtue of which the land area get extended into the water front and they play the role of 'land builders'. The soil thus conserved by the mangrove is very significant and they are the nursery grounds for finfishes and shellfishes. Production of wood, mainly for fuel is an important benefit getting from mangroves. Leaves are valued greatly as a cattle fodder. Tannin derived from the bark of mangroves has variety of uses, such as in the manufacture of ink, plastic and glue. It is also used for leather and fishing net preservation. Thus the mangroves are very helpful to common people. Kathiresan and Shigayuki (1999) found that of all the mangroves studied *R.mucronata,* Lam. showed the strongest anti-fungal activity. The tannins obtained from the bark exhibited higher anti-fungal activity than those from leaves and wood. Premanathan *et al.,* (1996) were screened the mangrove plant extracts in vitro against human immunodeficiency virus (HIV) on MT- 4 cells and found that the plants which belong to the family Rhizophoraceae are more active.

A. VEGETATION STUDY

The taxa collected were identified using standard floras (Hooker, 1885; Gamble, 1915 -1936; Matthew, 1983). The herbarium of the plants

TABLE - 1. NAME OF THE TAXA INVESTIGATED FROM PUDUVYPPU AND VALLARPADAM

Sl. No.	Name of Taxa	Family
	MANGROVES	
1.	*Bruguiera cylindrica*, W. & A.	Rhizophoraceae
2.	*B. gymnorrhiza*, Lam.	,,
3.	*Rhizophora mucronata*, Lam.	,,
4.	*Acanthus ilicifolius*, L.	Acanthaceae
5.	*Avicennia officinalis*, L.	Avicenniaceae
6.	*Excoecaria agallocha*, L.	Euphorbiaceae
7.	*Acrostichum aureum*, L.	Polypodiaceae
	MANGROVE ASSOCIATES	
8.	*Calophyllum inophyllum*, L.	Guttiferae
9.	*Hibiscus tiliaceus*, L.	Malvaceae
10.	*Thespesia populnea*, Cav.	,,
11.	*Cayratia pedata*, Juss.	Vitaceae
12.	*Aschynomene aspera*, L.	Papilionaceae
13.	*A. indica*, L.	,,
14.	*Derris trifoliata*, Lour.	,,
15.	*Terminalia catappa*, L.	Combretaceae
16.	*Eclipta alba*, Hassk.	Compositae
17.	*Mikenia scandens*, Willd.	,,
18.	*Sphaeranthus indicus*, L.	,,
19.	*Wedelia trilobata*, (L.) As. Hith.	,,
20.	*Cerbera odollam*, Gaertn.	Apocynaceae
21.	*Ipomoea companulata*, L.	Convolvulaceae
22.	*I. palmata*, Forsk.	,,
23.	*I. sepiaria*, Koen.	,,
24.	*Hygrophila angustifolia*, R.Br.	Acanthaceae
25.	*Clerodendron inerme*, Gaertn.	Verbenaceae
26.	*Premna latifolia*, Roxb.	,,
27.	*Alternanthera sessilis*, (L.) R.Br.	Amaranthaceae
28.	*Antidesma ghaesembilla*, Gaertn.	Euphorbiaceae
29.	*Synostemon bacciforme*, (L.) Web.	,,
30.	*Cleome burmanni*, W.&A.	Capparidaceae
31.	*C. viscosa*, L.	,,
32.	*Sida acuta*, Burm.	Malvaceae
33.	*S. cordifolia*, L.	,,
34.	*Urena lobata*, L.	,,
35.	*Corchorus acutangulus*, Lam.	Tiliaceae
36.	*Triumfetta rhomboidea*, Jacq.	,,
37.	*Desmodium dichotomum* (Willd.) DC.	Papilionaceae
38.	*Ammania baccifera*, L.	Lythraceae
39.	*Mukia scabrella*, Arn.	Cucurbitaceae
40.	*Ludwigia parviflora*, Roxb.	Onagraceae
41.	*Borreria hispida*, K. Sch.	Rubiaceae
42.	*Oldenlandia herbacea*, (L.) Roxb.	,,

43.	*O.umbellata*, L.	,,
44.	*Ageratum conyzoides*, L.	Compositae
45.	*Blumea lacera*, (Burm.f.) DC.	,,
46.	*Emilia sonchifolia*, DC.	,,
47.	*Synedrella nodiflora*, Gaertn.	,,
48.	*Vernonia cineria*, Less.	,,
49.	*Lobelia alsinoides*, Lam.	Companulaceae
50.	*L. trigona*, Roxb.	,,
51.	*Physalis minima*, L.	Solanaceae
52.	*Scoparia dulcis*, L.	Scrophulariaceae
53.	*Justicia gendarussa*, L.	Acanthaceae
54.	*Lippia nodiflora*, Mich.	Verbenaceae
55.	*Stachytarpheta indica*, (L.) Voht.	,,
56.	*Hyptis suaveolens*, Poit.	,,
57.	*Boerhaavia diffusa*, L.	Nyctaginaceae
58.	*Achyranthes aspera*, L.	Amaranthaceae
59.	*Euphorbia hirta*, L.	Euphorbiaceae
60.	*Phyllanthus niruri*, L.	,,
61.	*Murdannia nudiflora*, (L.) Brenan.	Commelinaceae
62.	*Cyperus iria*, L.	Cyperaceae
63.	*Fimbristylis acuminata*, Vahl.	,,
64.	*F. dichotoma*, Vahl.	,,
65.	*Fuirena ciliaris*, (L.) Roxb.	,,
66.	*Kyllinga brevifolia*, Rottb.	,,
67.	*K. polyphylla*, Willd. Ox Kuntu.	,,
68.	*Pycerus polystachyus*, Beauv.	,,
69.	*Scleria laevis*, Retz.	,,
70.	*Chloris barbata*, Sw.	Gramineae
71.	*Dactylactenium aegyptium*, Beauv.	,,
72.	*Eleusine indica*, Gaertn.	,,
73.	*Panicum maximum*, Jacq.	,,
74.	*Paspalum scrobiculatum*, L.	,,
75.	*Zoysia matrella*, Merr.	,,

Classification according to Bentham and Hooker.

studied were prepared and voucher specimens housed at the herbarium of Department of Botany, St. Teresa's College, Ernakulam.

The list of taxa investigated with their families is given in Table -1. The plants collected are arranged according to the classification of Benthem and Hooker.

B. FOLIAR EPIDERMAL STUDIES

Following the methods of Inamdar and Patel (1969) imprints of epidermis were taken to study the development of stomata. Fresh, young and delicate leaf materials of which the peels were not easy to get, imprints of the epidermis were taken by using a transparent adhesive mountant. The adhesive used are Fevicol, transparent nail polish and DPX mountant.

Stomatal ontogeny

Edwards (1935) was the first to make a detailed systematic survey of cuticular studies in angiosperms. The diacytic stomata of Acanthaceae have been widely accepted as a conservative taxonomic character (Ahmad, 1972, 1979 b; Inamdar *et al.*, 1983; Stace, 1989), as the stomatal organisation is unmistakably constant throughout the family.

Stomatal Index and Stomatal Frequency.

These are calculated by the method suggested by Salisbury (1927, 1932).

The peelings of the fresh leaves were taken by direct peel method and stained in safranin and observed under the microscope. With the aid

of prism type camera lucida, the number of epidermal cells and stomata in the field was noted. Stomatal index and stomatal frequency were calculated using the following formula:

Stomatal Index (SI) = S/S+E×100, where,

E = Number of epidermal cells in the field

S = Number of stomata in the field

Stomatal Frequency (SF)

$SF/mm^2 = XY^2/S \times 10^6$ where,

X = Number of stomata in the field

Y = Magnification

S = Square area of the field

Palisade Ratio

Clearing technique was another method used for the study of epidermal features. Leaves were cleared using 70% Chloral hydrate or Trichloro acetic acid and Phenol in the ratio (2:1). The palisade ratio was determined by counting the number of palisade cells under four adjacent epidermal cells from five different leaves of the same plant. From the mean value of this count, the ratio of the palisade cell to the epidermal cell was found out.

PR = Number of palisade cells/ Number of epidermal cells (Epidermal cells a constant = 4).

Preparation of peelings for Histochemical studies

Foliar micro-morphological studies were done using fresh specimens. From fresh leaves, epidermal peelings were taken by direct peel method, stained in Saffranin or Toluidine blue and mounted in glycerine. Peelings from thick leaves with a close network of veins were obtained by scrapping off all the unwanted tissue using a sharp edged scalpel and forceps. For the study of stomatal index, stomatal frequency and palisade ratio, the micro-preparations were mounted in pure glycerine. However, Iodine-potassium iodide solution used for the detection of starch was found to be a very useful semi-permanent mounting medium, especially for recording the number of starch grains.

C. HISTOCHEMICAL ANALYSIS

a. Qualitative - Histochemical localisation of metabolites in foliar epidermis.

For this study, fresh materials alone were used. To get uniform results, variables like time, temperature and concentration of the stains were kept constant. The methodologies adopted by Johansen (1940), Jensen (1962), Krishnamurthy (1988) and Vijayaraghavan and Shukla (1990) were followed.

The metabolites localised and the methods adopted were:

1. Starch -- Iodine-potassium iodide reaction (Johansen, 1940)

The peelings of the leaves were mounted in Iodine-Potassium iodide solution made by dissolving 2g of Potassium iodide in 100ml of water and

then mixing 0.2g of iodine in the potassium iodide solution. The blue-black colour indicated the presence of starch.

2. **Insoluble polysaccharides -- Periodic acid-Schiff's reaction or PAS reaction** (Mc Manus, 1948)

The epidermal peels of leaves were taken and oxidised in 1% periodic acid for 10 minutes. They were washed thoroughly in running water to remove the periodic acid. Then the peels were placed in Schiff's reagent for 20 minutes in darkness, washed in water and mounted in glycerine. The purplish red colour so developed indicated the presence of insoluble polysaccharides.

3. **Sulphated and Carboxylated polysaccharides -- Toluidine Blue O method** (O'Brien *et al.*, 1964; Feder and wolf, 1965; Mc Cully, 1966)

Fresh peelings were taken and stained in TBO (0.05% Toluidine blue O in Benzoate buffer) for five minutes. Then the excess stain was washed away in running water and mounted in glycerine. Sulphated and carboxylated polysaccharides stained pink to reddish pink.

4. **Polyphenols -- Toluidine blue O method** (O' Brien *et al.*, 1964; Feder and Wolf, 1965; Mc Cully, 1966).

The epidermal peelings were taken and stained (0.05% Toluidine blue O in Benzoate buffer) for five minutes in the stain solution. The excess stain was washed away in running water and mounted in glycerine. Polyphenols stained turquoise blue.

5. Total Proteins – Mercuric Bromophenol Blue method (Mazia *et al.*, 1953; Ruthmann, 1970; Chapman, 1975).

The peelings were immersed in the dye solution for 15 minutes (10g of Mercuric chloride and 100mg of Bromophenol blue were dissolved in 100ml of water). Washed for 20 minutes in 0.5% acetic acid to remove excess stain. Washed in water for 15 minutes and mounted in glycerine. Blue-brown colour developed indicated protein.

6. Total lipids -- Sudan Black Dye method (Baker, 1947, Gomori, 1952).

Fresh epidermal peels of leaves were taken and pre-treated with 70% ethanol and stained with freshly prepared Sudan Black dye (0.4g Sudan Black dye in 70% Ethanol). Rinsed in ethanol for 1 minute, washed in water and mounted in glycerine. Lipids and lipoproteins were stained black.

Histochemical results were photographed with NIKON HFX trinocular research microscope with NIKON – FX – 35 A camera. Neutral density filter was used to control light rays from the camera. KONICA super SR-100 colour films were used for histochemical micrographs.

b. Quantitative

The quantitative estimation of the various metabolites in the leaves of the plants was conducted. Leaves from 3rd or 4th position from shoot apex were used for extraction. Spectrophotometer readings were employed for the study. The period of investigation was divided into three seasons *viz.* pre-monsoon, monsoon and post monsoon.

1. Chlorophyll and Carotenoid contents

The method of Arnon (1949) was employed for the quantitative estimation of chlorophyll and carotenoid contents.

80% acetone was prepared and a pre-weighted (250mg) quantity of fresh leaf material was ground into a fine paste. 10 ml of acetone was added into it. The extract was centrifuged and the green supernatant was obtained. Using small quantities of acetone, the extract was centrifuged repeatedly till the lechate became colourless. The supernatant was taken together and was made up to 25ml with 80% acetone. The extract was kept away from direct sunlight. The optical density of the extract was read at different wavelengths such as 480, 510, 645, 652 and 663nm. The samples were analysed in duplicate. From the optical densities, the chlorophyll *a*, *b*, total chlorophyll and carotenoid contents were calculated.

2. Total Carbohydrate content

For the quantitative estimation of carbohydrate, the method adopted was that of Shirlaw and Gilchrist (1967). This was based on spectrophotometer observations.

One gram each of Anthrone and Thiourea were taken. 760ml of concentrated sulphuric acid was added to 240ml of distilled water. Anthrone and Thiourea were dissolved in this.

200mg of fresh dried ground leaf samples were taken and boiled half-an-hour with 20ml of distilled water in a 250ml conical flask. It was filtered and the volume was made up to 50ml with distilled water. 1ml of

the filtrate was pipetted in a test tube and 10ml of Anthrone reagent was added. After stoppering the test tubes with rubber plugs, they were kept in water bath at 100°C for 20 minutes. Test tubes were removed from water bath and were cooled in running water. The colour density developed was measured spectrophotometrically at 625nm.

Preparation of standard carbohydrate solution:

Dissolving 100mg glucose in distilled water and making it up to 1000ml prepared a standard carbohydrate solution of 100ppm concentration.

3. Total Protein (Lowry method - Lowry *et al.,* 1951).

The fresh dried leaf samples were prepared and 200mg of the leaf powder was weighed out. This was extracted with 10ml of 0.1N NaOH for 12 hours. By centrifugation, the supernatant was obtained and assayed by the method of Lowry.

Reagents for Lowry method:

Reagent A - 2% Na_2CO_3 in 0.1N NaOH.

Reagent B - 0.5% $CuSO_4$ .5 H_2O in 1% Sodium or Potassium tartarate.

Reagent C - Mixed 50ml of reagent A and 1ml of reagent B.

Reagent D - 1 part phenol reagent and 2 parts distilled water.

0.5ml of protein sample was combined with 5ml of reagent C and mixed well. It was allowed to stand for 10 minutes at room temperature. Then rapidly added 0.5ml. of reagent D and mixed immediately and allowed to stand at room temperature for 10 minutes. Read at 600nm in the spectrophotometer.

Bovine serum albumen was used as the standard.

4. Tannins. (Folin - Denis method: Schanderl, 1970)

Weighed 0.5g of fresh dried powdered leaf sample was transferred to a 250ml conical flask. Added 75ml water and heated the flask gently and boiled for 30 minutes. Centrifuged at 2000rpm for 20 minutes and collected the supernatant in 100ml volumetric flask and make up the volume.

Reagents for Folin - Denis method

Dissolved 100g Sodium tungstate and 20g Phosphomolybdic acid in 750ml distilled water in a suitable flask and added 50ml Phosphoric acid. Refluxed the mixture for 2hours and make up to 1 litre with distilled water. Protect the reagent from exposure to light.

Dissolved 350g of Sodium carbonate in 1 litre of distilled water at 70-80°C. Filtered through glass wool after allowing it to stand overnight.

Transferred 1ml of the sample extract to a 100ml conical flask containing 75ml water. Add 5ml of Folin - Denis reagent, 10ml of sodium carbonate and diluted to 100ml with distilled water. It was shaken well and read at 700nm after 30 minutes in the spectrophotometer.

Tannic acid solution was used as the standard.

5. Moisture

Fresh leaves from 7 species of mangrove plants, locally available in the Cochin backwaters were selected for this study. Actively photosynthesising green leaves of the plants were collected and

transported to the laboratory in polythene bags without adding any preservative. The initial weight of the collected samples were determined and then kept in the oven at 50°C. After drying to constant weight, the final weight was also noted, from which the moisture content was calculated.

6. Ash

The ash content of the dry samples was estimated by incinerating the plant material at 550°C for six hours in a muffle furnace and the ash samples were saved for the determination of minerals.

7. Nitrogen

The nitrogen content in the leaves was estimated by Kjeldhal method.

8. Sodium and Potassium

The ash was dissolved in (1-3 v/v) hydrochloric acid with a few drops of 70% nitric acid, boiled, cooled and then made up to 250ml. in a volumetric flask (AOAC, 1965).

The above extract was used to find out the quantity of sodium and potassium contents using their respective filters in a TMF 45 Flame photometer.

D. POLLEN MORPHOLOGY

Palynology is one of the most applicable scientific branches, both in pure and applied fields of natural sciences. Pollen grains are produced in the anther–the male reproductive part of flowering plants. They carry the

genetic material from one generation to the other. The most striking feature of the pollen grain is that, its structure and sculpturing make it a highly recognizable object. The relevance of pollen morphology as an index in cultivar taxonomy has been amply demonstrated with regard to several cultivated plants (Nair, 1961, 1965).

The shape of the pollen grains is calculated by taking measurements of pollen axis (P) and the maximum breadth (E) in the equatorial view of the grain and applying the formula - P/E X 100. The value obtained gives the shape. The maximum breadth is mostly identical with the equatorial diameter and based on P/E ratios, there are nine different classes (Erdtman, 1952).

The size of pollen grains in modern flowering plant varies from 5 x 2.4 μm to 200μm or slightly more. The size is usually represented as the length of the longest axis. Walker and Doyle (1975) proposed six classes based on pollen size, ranging from minute grains to gigantic ones.

Most pollen grains possess openings or thin parts on the exine through which the pollen tubes emerges out. Two types of apertures are commonly observed - the pores and furrows or colpi. The difference between furrows and pores seems to be purely morphological: furrows are elongate or boat shaped with acute or blunt ends, while the pores are isodiametric. Phylogenetically, the furrows are the primitive form and pores are developed later by contraction.

Preparation of pollen grains for the morphological studies:

Both light microscopic and scanning electron microscopic studies were done for the detailed analysis of pollen grains. Mature anthers of unopened flower buds were collected for the pollen grains (to minimise variations) and preserved in 70% ethanol. They were acetolysed by the method of Erdtman (1952), Nair (1974) and mounted in glycerine jelly and examined under light microscope.

Photography:

For scanning electron microscopic (SEM) study, pollen grains were gathered from mature, indehiscent anthers, mounted on stubs using double-sided adhesive tape, coated with gold in vacuum coater and viewed with Hitachi S-450 stereo scan and photographed. The photographs were recorded in INDU-120 black and white films. The negatives were printed on AGFA professional RC glossy paper in AGFA Modern 2-2B enlarger. For histochemical microphotographs Konica super SR-100 colour films were used. These were taken in NICON HFX trinocular microscope with NICON - FX - 35A camera. Neutral density filter was used to control light rays from the source.

Drawings:

All drawings pertaining to this study were made using prism type camera lucida at table level. The diagrams were drawn at varying magnifications depending on the materials and nature of study.

E. HYDROLOGICAL PARAMETERS

Collection of Samples:

Monthly sampling of surface water was made regularly from five different sites in each station for estimating the hydrological parameters like temperature, pH, salinity, dissolved oxygen, and the productivity of the water, in which the mangroves were growing. Water samples were collected for the analysis in narrow mouthed airtight transparent plastic bottles without any air bubbles before 9.00hr in the morning. As in the case of dissolved oxygen estimation, the initial bottles were fixed on the spot by Winkler A and B. To keep uniformity, 5 hrs of incubation were given every time for the light and dark bottles in the field. The samples thus fixed (initial, dark and light bottles) were transported carefully to St. Teresa's college, laboratory for further analysis and estimation.

A number of factors affect the structure and productivity of mangrove forests including hydrology, nutrient inputs, soil salinity and soil type (Lugo *et al.*, 1988), seasonal changes in rainfall, soil salinity, air temperature, water temperature, nutrient inputs and water turnover rate (Twilley *et al.*, 1986; Robertson and Alongi, 1993; Day Jr. *et al.*, 1996). The continual mixing of water masses complicates understanding the hydrography and nutrient cycling in the mangrove water.

Cochin backwater is a positive type of estuary and the hydrographic profile is governed by tidal incursion, evaporation and the

run-off from the rivers emptying into the backwater, which make it an extremely unstable ecological system.

1. Temperature:

More than any other hydrological factors of the marine environment, the temperature controls growth, reproduction and distribution. Temperature has direct effect on basal metabolic rate. Increased metabolic rate accelerates growth and make faster sexual maturity of the organisms.

The temperature of the surface water samples was measured at the site soon after collection of water sample with an accuracy of ± 0.1°C using precision thermometer (0-50°C).

2. pH (hydrogen ion concentration)

pH was measured with the help of a pH meter.

3. Salinity:

Salinity was estimated by using a conductivitimeter.

4. Dissolved oxygen:

For the determination of DO, standard Winkler method was used (Strickland and Parsons, 1968).

To the water samples in 250ml BOD bottles, 2ml of manganous sulphate solution and 2ml of alkali iodine azide solution were added. The solution was mixed thoroughly and the precipitate was allowed to settle. Later, this precipitate was dissolved by adding 2ml of concentrated sulphuric acid and was shaken well, till the precipitate dissolved

completely. From this solution, 100ml was taken into a 250ml conical flask and titrated with standard 0.1N sodium thiosulphate solution till a pale straw colour developed. A few drops of starch indicator solution were added until the solution turned blue. Titration was continued till the blue colour disappeared. From the titre values, the concentration of dissolved oxygen was determined.

5. Primary Production:

Monsoon plays a critical role in triggering environmental features such as seawater temperature, salinity, dissolved oxygen content and nutrient generation, which in turn become responsible for production of phytoplankton and zooplankton. At Cochin, the levels of gross primary production showed increase from 0.830 to 1.624gC/m^2/day corresponding with increasing rainfall during April to June (Rajagopalan *et al.*, 1992). The annual net primary production in the euphotic waters of Cochin was estimated as 731.43 tonnes Carbon/km^2 indicating high productivity. Vegetation dynamics and primary production in saline, lacustrine wetlands was studied by Andrew and James (2000) and leaf primary production in *Posidonia oceanica* (sea grass) was carried out by Paolo, 2000.

6. Gross Primary Production:

Oxygen technique (Gaarder and Gran, 1927) was used for the estimation of primary productivity. In this method, the bottles were categorised into light and dark. In the dark bottles, only respiration takes

place, where as in the light bottle both photosynthesis and respiration take place. The difference in the oxygen content between light and dark bottle was taken for estimating the gross primary productivity and expressed in mgC/l/ day.

GPP = L-D× 0.536/1.25 × T where, L and D are the dissolved oxygen in the light and dark bottles, 1.25 is the photosynthetic quotient, T is the incubation time in hours (5hrs) and 0.536 is a constant.

7. Net Primary Production:

For estimating the net primary productivity, the difference in the oxygen content between light and initial was taken and expressed in mgC/l/day.

NPP = L-I × 0.536 / 1.25 × T where, L and I are the dissolved oxygen in the light and initial bottles, 1.25 is the photosynthetic quotient, T is the incubation time in hours (5hrs) and 0.536 is a constant.

F. ECONOMIC IMPORTANCE:

Mangroves are remarkable tropical plants of great economic significance. The mangrove forests provide native populations with a seemingly endless variety of derived products: timber, thatching materials, charcoal, medicines, animal fodder *etc.* (De La Cruz, 1979). The mangrove environment also yields an abundant supply of fish and prawns from its waterways, shellfish such as oysters and crabs from the shore zone and birds eggs, honey and edible fruits from forest areas (Macintosh, 1982). Prawn rearing in configuration with paddy cultivation is carried out in the

coastal 'bheries' of West Bengal and in Cochin backwaters (Menon, 1954; George *et al.*, 1968; Jhingran, 1975; Kurian and Sebastian, 1976). Mangroves also act as an important source of seed for commercially important prawns and fishes (Achuthankutty and Sreekumaran Nair, 1980; Bopiah and Neelakandan, 1986; Achunthankutty, 1990; Vance *et al.*, 1990).

The very important environmental service provided by the mangroves is that they build land or keep it from being washed away. The roots and trunks break the force of the waves, and the leaves and branches reduce the effects of the wind and rain.

Ecologically mangroves are important in maintaining and building the soil, as a reservoir in the tertiary assimilation of waste, and in the global cycle of carbon dioxide, nitrogen and sulphur. Mangroves play a significant role in coastal stabilization and promoting land accretion, fixation of mud banks, dissipation of winds, tidal and wave energy.

The uses of mangroves are many and varied. They are used in flavouring agents, textiles, mats, paper, housing, baskets, boats and tapa cloth and also used as staple food. The tender leaves of *A.aureum* and the hypocotyl of *Bruguiera* are the staple food of some Papua New Guineans. The importance of bark tannin has declined in many Asian countries but mangrove tannin is still used in India and Bangladesh for leather curing and in Sri Lanka tannin is used traditionally in curing fishnets. Mangrove plants are rich sources of saponins, alkaloids and flavonoids.

Wetlands perform some important function like flood control, water storage, protection of shorelines and hinterlands, floral and faunal habitats and gene pools besides providing outputs of commercial value and economic sustenance to the people. Several voluntary organisations started working by regeneration of forests through active involvement of the natives.

A non-governmental organization, the International Society for Mangrove Ecosystem (ISME), which was established in 1990, aims to promote the study of mangroves with the purpose of enhancing their conservation, rational management and sustainable utilization.

Habitat protection is the ultimate goal of conservation, to which all other approaches are subsidiary. For conservationists world wide, mangroves present the great immediate challenge.